

## Generating chemocompetent cells

- Material:
  - 300 ml **LB-Medium**
  - ~10 ml cooled TSS buffer
  - 5 pre-cooled 50 ml Falcons
- Protocol:
  - Inoculate 2x3 ml LB with bacterial stock; incubate over night at 37 °C and 200 rpm
  - Inoculate 1x250 ml LB with the over night cultures in 1 ml flask at 37 °C and 140 rpm
  - Incubate until  $OD_{600}$  0.4-0.6
  - Devide the culture into five cooled 50 ml Falcons and incubate on ice for 10 minutes
  - Onwards all steps at 4 °C
  - Centrifugate at 3000 rpm, 4 °C for 10 minutes
  - Discard supernatant
  - Pipette out remaining media
  - The volume of **TSS** to use is 10% of the culture volume that you spun down (we only used 1-2 ml). You may need to vortex gently to fully resuspend the culture, keep an eye out for small cell aggregates even after the pellet is completely off the wall.
  - Use 100 µl per aliquot and drop them in liquid nitrogen immediatly
  - Store at -80 °C

